Polyploidy in angiosperms

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Polyploidy has played a major role in higher plant evolution. Most flowering plants are polyploid, and many are distinct in combining the diploid nuclear genomes from two or more different ancestral species or genera (allopolyploids). Recently, molecular techniques have offered powerful new tools for studying the origin and evolution of polyploids. Genomic *in situ* hybridization allows unequivocal identification of allopolyploids and visualization of their ancestral genomes. Studies of restriction fragment length polymorphism have shown that maize – hitherto generally viewed as a diploid – is really a tetraploid, and that multiple origins are common, if not the rule, for polyploid plant species. It appears that after polyploid formation, considerable and sometimes very rapid changes in genome structure and gene expression have occurred.

Polyploids have three or more complete sets of chromosomes in their nuclei instead of the two found in diploids (Box 1). The level of ploidy can be extremely high. The stonecrop *Sedum suaveolens*, which has the highest chromosome number of any angiosperm ($2n$ of about 640, where n is the gametic chromosome number), is estimated to be about 80-ploid[1]. Unlike in animals, where polyploidy is relatively rare, it has been estimated that 95% of pteridophytes and up to 80% of angiosperms are polyploid[2]. Thus polyploidy has played a significant role in the evolution of higher plants, although its occurrence is surprisingly uncommon in gymnosperms and highly variable between different angiosperm families. A particularly distinctive feature of the evolution and speciation of flowering plants is allopolyploidy – the combination of genetically different diploid nuclear genomes from two or more different ancestral species or genera. Understanding polyploidy assumes even greater significance when it is considered that most important crops are polyploid (e.g. wheat, maize, sugar cane, potato, coffee and cotton).

Novel uses of molecular techniques have recently opened up powerful ways for investigating the genomic or DNA sequence constitution of polyploids. These provide exciting new insights into the molecular events that have occurred during polyploid evolution. This review considers the impact of these new findings for understanding polyploidy in angiosperms.

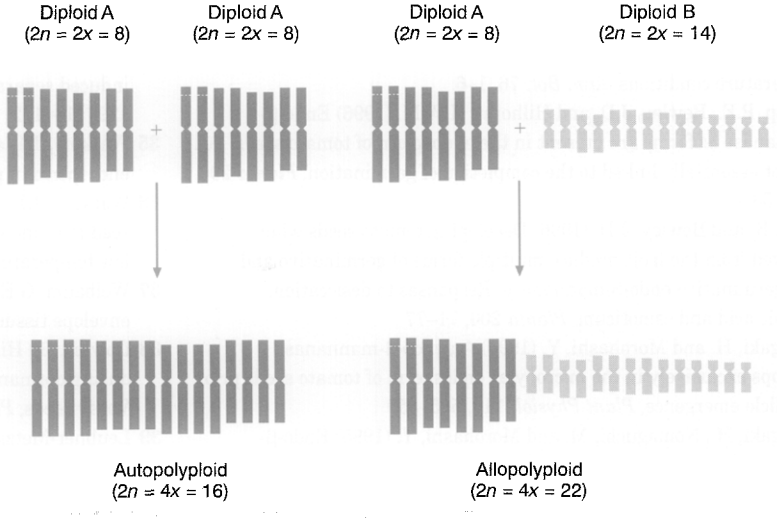
Molecular methods for analysing polyploids

Numerous molecular techniques have been exploited to investigate polyploid genome organization and evolution. Molecular mapping, including restriction fragment length polymorphism (RFLP) analysis, has been invaluable for monitoring the linear organization and distribution of DNA in the chromosomes, and has contributed to the identification of new polyploid species based on comparative mapping. RFLP analysis of both chloroplast and

Box 1. What is a polyploid?

Polyploids contain more than two genomes:

- A genome is the total DNA in one basic set of chromosomes (x), as found in a mature pollen or egg cell nucleus prior to fertilization (n is the gametic chromosome number)
- A diploid cell nucleus contains two genomes, as found in egg cells following fertilization.
- A polyploid cell nucleus contains more than two genomes. Two basic types of polyploids are recognized: autopolyploids, which contain more than two genetically identical genomes; and allopolyploids, which combine genomes from more than one ancestral species (e.g. an allotetraploid contains four genomes from two different diploid ancestral species).



In nature, auto- and allopolyploids are not always clearly recognizable, and a whole spectrum exists with many polyploids falling somewhere in between these two extremes. The identification of a polyploid of unknown genome composition is often difficult, because many polyploids [e.g. hexaploid wheat (*Triticum aestivum*)] are known to behave cytogenetically as diploids, with strict bivalent pairing and recombination at meiosis. Although the genes responsible for this have been mapped in some cases [e.g. the *Ph1* ('pairing homologous') in wheat] their precise mode of action is still unknown.

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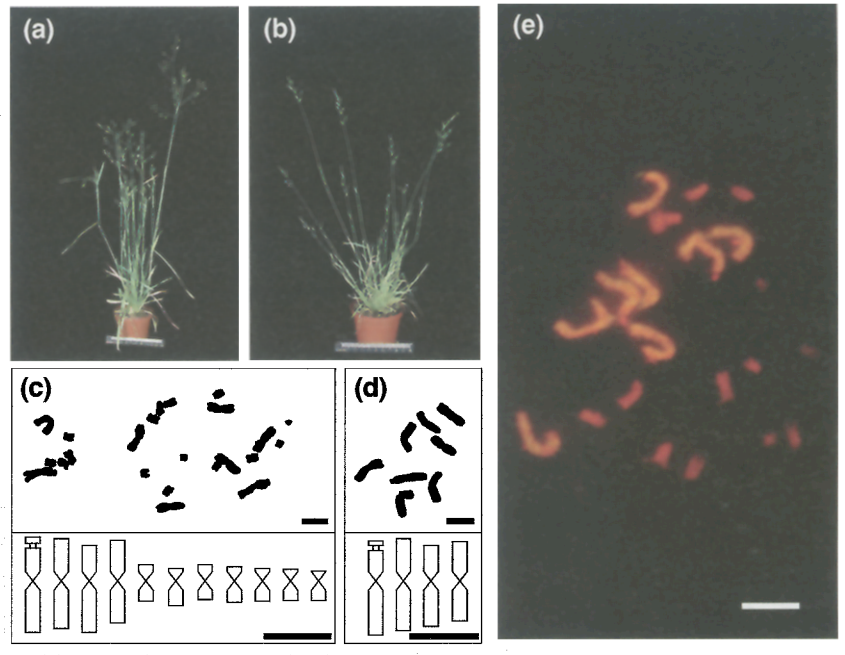
ribosomal DNA sequences has also been used for identifying the parental origin of polyploid genomes, and has provided compelling evidence that polyploids have multiple origins.

The development of genomic *in situ* hybridization (GISH) has provided new insights into the origin and evolution of polyploid genomes. GISH combines conventional cytogenetics with DNA–DNA *in situ* hybridization techniques, allowing chromatin of different parental or ancestral origins to be distinguished. The first use of GISH for analyzing a wild, naturally occurring polyploid was in a study to test the genomic origin of, and relationship between, the grasses *Milium montianum* and *M. vernale*[3] (Box 2). The results revealed that *M. montianum* is an allopolyploid and suggested that one of the genome donors was *M. vernale* or some close relative. They also showed that *M. montianum* is genetically distinct from *M. vernale* and thus should be given full specific status. This biosystematic use has developed rapidly and GISH is now widely applied to allopolyploids to reveal their genomic origin and organization[4].

Box 2. Testing the genome origins of *Milium montianum* using genomic *in situ* hybridization



Based on morphology, the grass *Milium montianum* (a) was originally recognized as a distinct species separate from *M. vernale* (b). Cytological analysis of plants that proved on morphological grounds to be *M. montianum* revealed a bimodal karyotype ($2n = 22$, where n is the gametic chromosome number) consisting of eight large and 14 small chromosomes (c). This was in contrast to the karyotype of *M. vernale* ($2n = 8$), which consisted of just eight large chromosomes (d). In (c) and (d), the upper panels show root-tip metaphase chromosome preparations and the lower panels show haploid idiograms (diagrammatic representations of chromosome morphology). The similarities in size, shape and number of the eight large chromosomes of *M. montianum* with those of *M. vernale* suggested that *M. montianum* may be an allotetraploid containing two genomes donated by *M. vernale* or some close relative. Conventional methods of resolving this question (i.e. making hybrids and analysing meiosis) failed, and thus genomic *in situ* hybridization was tried. When genomic DNA from *M. vernale* was used to probe chromosome spreads from *M. montianum*, it hybridized preferentially to all eight large chromosomes, labelling them yellow uniformly along almost their entire length (e). The small chromosomes were unlabelled, appearing red because of the counterstain. The result confirms that *M. montianum* is an allopolyploid, containing two genomes donated by *M. vernale* or some close relative, and shows that *M. montianum* is genetically distinct from *M. vernale* and thus should be taxonomically separated and given full specific status. Scale bar for (c), (d) and (e) represents 5 μm.

Evidence for new polyploid species

The recent upsurge in genome mapping initiatives and the identification of synteny (conservation of linkage groups between species) has shown that several crop species traditionally considered as diploid are actually polyploid. Comparative RFLP mapping of grasses has shown that maize, generally thought to be a diploid, has two distinct genomes[5] and is thus tetraploid (Fig. 1). Similarly, genome mapping of modern species of *Brassica*, which are widely considered to be diploid, has identified extensive stretches of DNA present in three collinear copies compared with the related diploid species *Arabidopsis*. This suggests that modern species of *Brassica* have descended from a common hexaploid ancestor and are thus degenerate hexaploids[6]. Extending comparative mapping to other Crucifers, including *Crambe*, *Sinapis* and *Moricandia*, suggests that they too share the common hexaploid ancestor with *Brassica* and thus may also all be hexaploids.

Genome mapping has also revealed that the level of ploidy may have been underestimated for some species already recognized as polyploids. For example, cotton has traditionally been considered to be allotetraploid ($2n = 4x = 52$, where x is the ploidy level). However, the RFLP map made by Reinisch *et al.*[7] indicates that it is a paleo-octoploid formed perhaps 1.1–1.9 million years ago following a polyploidization event that converted the basic chromosome number from 13 to 26.

Multiple origins of polyploid species

An exciting recent development is the discovery that a single polyploid species can originate more than once (i.e. it has multiple origins[8]). This challenges the long-held view that polyploid formation is rare, with each polyploid species typically thought to have had a single origin. Although isozyme data provided some evidence for multiple origins of polyploid ferns[9], in many cases the data were ambiguous. Now, the use of molecular markers from chloroplast and

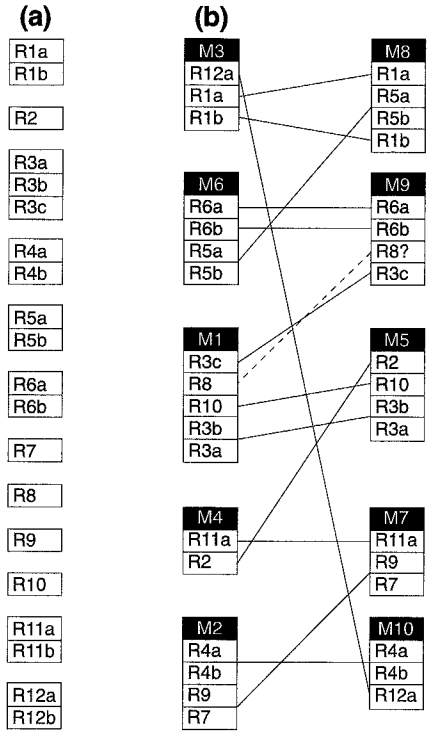


Fig. 1. The tetraploid structure of maize relative to rice. Restriction fragment length polymorphism mapping studies have divided the rice chromosomes into linkage blocks as shown in (a). Comparative mapping between rice and maize reveals that the maize chromosomes can be represented as rice blocks on the basis of homology and/or conservation of gene order. For example, maize chromosome M3 contains segments that are homologous to rice linkage blocks R12a, R1a and R1b (b). Connecting lines indicate duplicated segments within the maize chromosomes (e.g. a line connects rice linkage block R12a on maize chromosome M3 with a duplicated segment on maize chromosome M10), providing clear evidence that maize is a tetraploid with nearly all rice linkage blocks represented twice in the maize genome. The dotted line connects regions of uncertain homology. The ten maize chromosomes are arranged into two columns, with each column containing one haploid maize genome comprising five chromosomes. *Modified from Ref. 5.*

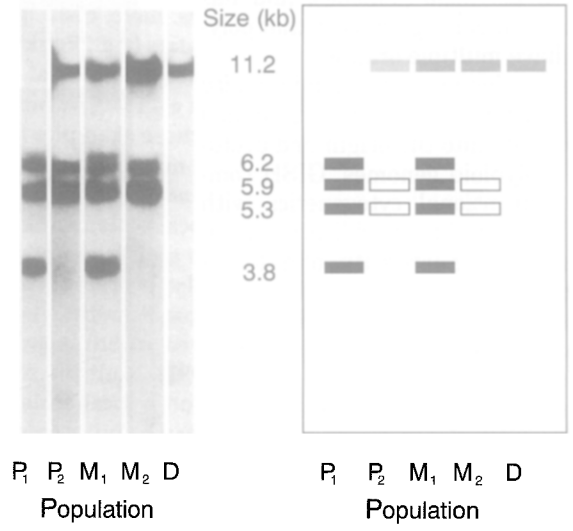


Fig. 2. Detecting multiple origins of the allotetraploid *Tragopogon mirus*. *T. mirus* contains two genomes from each of the diploids *T. porrifolius* and *T. dubius*. Restriction fragment length polymorphism (RFLP) analysis using ribosomal DNA (rDNA) as a probe revealed that all populations of *T. dubius* contained a single band of 11.2 kb (e.g. population D), which was present in all populations of *T. mirus* (e.g. populations M_1 and M_2). However, different populations of *T. porrifolius* contained different rDNA profiles. Population P_1 had fragment sizes of 6.2, 5.9, 5.3 and 3.8 kb, whereas population P_2 had fragment sizes of 11.2, 5.9, and 5.3 kb. An analysis of different populations of *T. mirus* showed that some (e.g. population M_1) contained the RFLP profile from population P_1 of *T. porrifolius*, whereas others (e.g. population M_2) contained the profile from population P_2, thus providing evidence of multiple origins of *T. mirus*. The data shown on the left are depicted on the right to show the parental origin of the different rDNA bands (e.g. black bands in M_1 lanes correspond to rDNA bands from population P_1 of *T. porrifolius*). *Modified from Ref. 10.*

ribosomal DNA has provided unequivocal evidence for multiple origins of polyploid species[10] (Fig. 2). Molecular markers have the distinct advantage over other markers (e.g. those based on biochemical and morphological characters) in that they are independent of gene expression; they are thus insensitive to the influence of environment and genetic background, and are developmentally stable.

Over 40 examples of multiple origins of both allo- and autopolyploids (Box 1) have now been documented in angiosperms, pteridophytes and bryophytes. Moreover, the data suggest that multiple origins are more common than single origins for polyploid taxa[11]. The highest demonstrated number of separate origins for a single polyploid species is 13 (e.g. in *Draba norvegica* and *Tragopogon miscellus*), but this value seems likely to increase.

From a systematic viewpoint, multiple origins of polyploids may offer one important explanation for taxonomically complex polyploid species, particularly where the probable diploid progenitor species have a wide geographical distribution. The genus *Draba* (Brassicaceae) is well known for its taxonomic complexity in arctic and alpine floras, and its polyploids, in particular, are difficult to group. The discovery that such polyploids have arisen recurrently from different populations of the same diploid progenitor species (polytopy) provides some explanation of the problems[12].

Polyploid genome evolution

There is increasing evidence to show that considerable and sometimes very rapid genomic change may occur following polyploid formation. Moreover, such changes have been identified at all levels of the genome, from the chromosome to the DNA sequence.

Chromosome-level evolution

GISH can not only identify allopolyploids and their parental genome donors (as in *M. montianum*; Box 2), but can also be used to examine the organization and evolution of the parental genomes in the polyploids. It is now clear that considerable structural rearrangements may occur

following polyploid formation. For example, GISH analysis identified nine intergenomic translocations in tobacco, which is allotetraploid[13]; it also revealed at least five intergenomic translocations in the allotetraploid *Avena maroccana*[14] and as many as 18 in the allohexaploid *A. sativa*[15]. RFLP analysis has also uncovered many chromosome interchanges in soybean, which is a tetraploid[16]. Similarly, although maize is now recognized to be tetraploid ($2n = 4x = 20$), the two 'genomes', each with five chromosomes, are differentiated such that no completely homologous chromosomes can be identified[5]. The maize genome is therefore no longer structured as a tetraploid, implying that considerable chromosomal reorganization has taken place since polyploidy first occurred. Gaut and Doebley[17] have shown that the extent of sequence divergence in the different duplicated segments of the maize chromosomes falls into two discrete groups. They propose that these differences correspond to different stages in the evolution of tetraploid maize.

The presence of intergenomic chromosome translocations in nature are particularly interesting in the context of polyploid evolution, because they represent new genomic arrangements that are possible only once a polyploid has formed. Jiang and Gill have distinguished two different types of intergenomic translocations[18]: 'random translocations', which occur in different chromosomes in different populations of the same polyploid species; and 'species-specific' translocations, involving specific chromosomes, and found in every polyploid population of a species. To explain the presence of the second type, Gill proposed the 'nucleo-cytoplasmic interaction (NCI) hypothesis' of genome evolution and speciation in polyploid plants[19]. According to this hypothesis, a newly formed polyploid must pass through a 'bottleneck' of sterility resulting from the adverse interaction between the male nuclear genome and the nuclear and cytoplasmic genomes of the female parent. Certain bottleneck (species-specific) chromosomal changes must occur in the nuclear genome to restore fertility and nucleo-cytoplasmic compatibility. Jiang and Gill[18] suggested that the intergenomic translocations they found in two tetraploid wheats belonged to the second type of chromosomal change, because they occurred in all populations examined.

Species-specific intergenomic translocations were also observed using GISH in allotetraploid tobacco (Fig. 3a), which combines two ancestral genomes known as the S and T genomes[13]. The S genome donor is widely accepted to be *Nicotiana sylvestris* or some close relative. The identity of the T genome donor is more controversial, although it is likely to come from Section Tomentosae. Kenton *et al.*[13] analysed three different tobacco genotypes and found that each line had up to nine different homozygous translocations between the S and T genomes (Fig. 3b). By physically mapping two highly repetitive DNA sequences, the identities of the chromosomes involved in three of the recombinants were determined, revealing at least one recombinant chromosome (ST1) common to all genotypes[20]. The chromosome ST1 may represent a species-specific chromosome translocation that occurred during polyploid formation and restored nucleo-cytoplasmic compatibility[19]. The continued physical mapping of DNA sequences and whole genomes by fluorescent *in situ* hybridization (FISH) should uncover other interesting types of chromosome rearrangements arising from polyploidy.

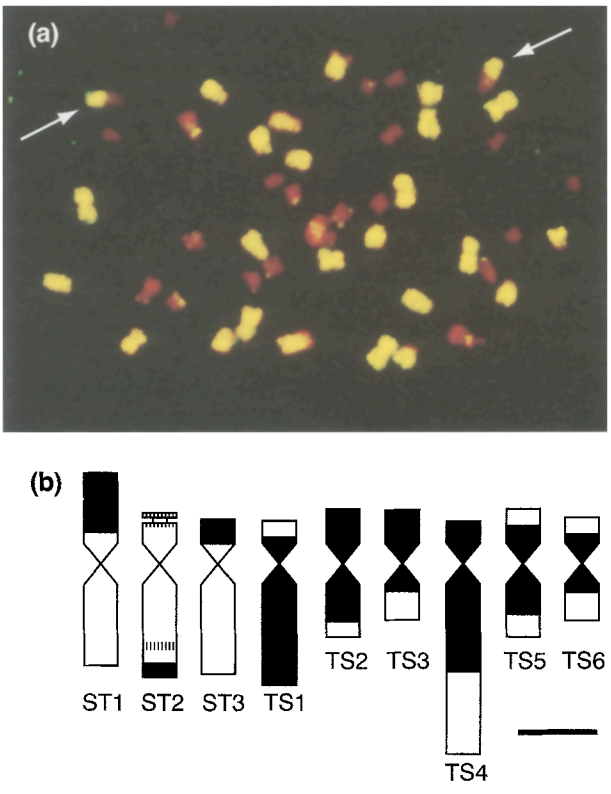


Fig. 3. Identification of intergenomic chromosome translocations in tobacco ($2n = 4x = 48$, where n is the gametic chromosome number and x is the ploidy level) using genomic *in situ* hybridization (GISH). (a) Chromosome preparations of tobacco cv. 095-55 probed with total genomic DNA from *Nicotiana sylvestris*, the S genome donor of tobacco. Sites of hybridization are visualized using the yellow fluorochrome fluorescein. Unlabelled chromosomes (from the T genome component of tobacco) appear red because of the counterstain. Two of the intergenomic translocations are arrowed. (b) Diagrammatic representation of the nine intergenomic translocations that have been identified in tobacco genotypes using GISH (Ref. 13). Black and white areas indicate T and S genome chromatin, respectively. Chromosome nomenclature follows that of Parokonny and Kenton[20]. Scale bar represents 10 μm in (a) and 1 μm in (b).

Sequence-level evolution

The bringing together of multiple genomes following polyploid formation also opens up the possibilities of intergenomic sequence interaction and evolution. Molecular studies are showing that similar sequences brought together from different genomes may undergo concerted evolution rather than continuing to evolve independently. The strongest evidence for this comes from work on *Gossypium* polyploids that have been assigned the genomic designation AADD (Ref. 21). The allotetraploids contain two ribosomal RNA (rRNA) gene loci from the A genome progenitor and two from the D genome progenitor. Analysis of the internal transcribed spacer (ITS) sequence between the 5.8S and 18S rDNA showed that the sequences had become identical to those of the D genome in most of the *Gossypium* allotetraploids. Interestingly, however, in the allotetraploid

G. mustelinum, the sequences homogenized to the A genome ITS sequence, indicating that interlocus evolution could be bidirectional. The mechanism of such interlocus homogenization was suggested to be unequal crossing-over that may be facilitated by the telomeric location of these rDNA sites in *Gossypium*. Currently, *Gossypium* is the only well-documented case of interlocus concerted evolution in angiosperms. However, Wendel *et al.*[21] pointed out that concerted evolution among rDNA loci may be more common than previously recognized given the accumulating evidence of ITS sequence homogeneity in 'diploid' plant groups suspected to be paleopolyploids.

Clearly, not all sequences or even all rDNA sequences are susceptible to concerted evolution. An analysis of 5S rDNA in allopolyploid cottons showed that the different loci had retained their subgenomic identity in the polyploid[22]. Interestingly, 5S rDNA sequences are located near the centromere in cotton chromosomes[23] and so may be unable to undergo unequal crossing-over and thus concerted evolution.

Song *et al.*[24] examined genome evolution in naturally occurring and synthetic *Brassica* polyploids. The extent of genome change was determined by following the fate of a variety of nuclear DNA clones using Southern hybridization. Although the nature of the DNA sequences being analyzed was mostly unknown, significant genome divergence was apparent in some of the polyploids. They concluded that the extent of change in the polyploid nucleus was, in part, determined by the degree of genome similarity between the different parental genomes of the polyploid. *Brassica* polyploids containing parental genomes from widely divergent diploid species underwent more extensive genomic change than those containing more similar parental genomes[24].

Song *et al.*[24] also claimed that the extent of genome change depended on parental origin. Thus, the genome originating from the maternal parent, which donates both the cytoplasm and the nucleus to the polyploid, underwent much less change than the genome from the paternal parent, which donates only its nuclear DNA to the polyploid. The greater genomic changes observed in the paternal genome presumably reflect the requirement for genome readjustments in a newly formed polyploid nucleus[19]. The analysis of synthetic *Brassica* polyploids showed that such genome change was rapid, with genome divergence already detectable only five generations after polyploid formation.

New patterns of gene expression in polyploids

The novel association of different species genomes within a single nucleus not only affects the evolution and organization of the DNA: the expression of the DNA itself may also be altered as a consequence of polyploidy.

Initially, isozyme analysis of polyploids provided some evidence of 'gene silencing', a process whereby the expression of additional copies of genes in the polyploid nucleus could be suppressed. For example, loss of duplicate gene expression of the isozyme leucine aminopeptidase was found in tetraploid *Chenopodium* species. The isozyme pattern reflected that found in the diploid ancestors of the polyploid[25]. More recently, DNA sequence analysis has shown gene silencing in several polyploid species[11]. If extensive, the process could reduce the level of gene expression to the diploid state. Thus, although the genome is polyploid with respect to the amount of genetic material and the number of gene copies it contains, it is essentially diploid with respect to the level of gene expression and chromosomal behaviour[26,27].

The mechanisms by which altered gene expression through gene silencing operate are still under debate. One suggestion is that silencing takes place by structural changes (e.g. insertions, deletions and nonsense mutations) in the DNA of the 'extra' gene copies, leading to defective, nonfunctional genes. For example, Pichersky *et al.*[28] identified many structurally defective copies of the chlorophyll *a*/*b*-binding (CAB) protein gene in a homosporous fern in addition to one functional *CAB* gene. Several hypotheses could explain this result, but the most likely explanation is that the defective genes represented the products of 'gene silencing' mechanisms following polyploidy.

Another suggestion centres on the observation that multiple copies of a sequence have been shown to trigger epigenetic phenomena, collectively termed homology-dependent or repeat-induced gene silencing. In plants, these can be divided into transcriptional silencing, probably reflecting regulation at the DNA level, and post-transcriptional silencing, which affects steady-state RNA levels. There is substantial evidence that the expression of foreign DNA introduced into plants can be reduced or lost in these ways[29,30]. The underlying molecular events are still poorly understood, but recent work implicates the possible involvement of specific gene sequences; repeat-induced changes in chromatin structure; the location of the sequence in relation to scaffold attachment regions; and/or cytosine methylation[31]. Similar mechanisms may operate on endogenous repetitive DNA sequences present in the plant genome (e.g. transposable elements and retroelements) that arise through replicative transposition of transposable elements or genomic turnover processes (e.g. unequal crossing over[32]). It is therefore possible that these processes could silence specific sequences present in multiple copies as a consequence of polyploid formation. Homology-dependent gene silencing does not operate on all types of repeated sequences (e.g. multigene families), and thus other mechanisms must exist to counteract or prevent it[33].

Evidence of extensive reduction in gene expression, through gene silencing, to that typical of a diploid has been provided in only a few polyploid plant species, including maize[11]. Here, many of the enzyme systems show expression patterns typical of a diploid[34], even though maize is now recognized as tetraploid. Increasingly, the genomes of ferns, which show diploid isozyme expression patterns, are thought to represent the products of ancient polyploid events followed by extensive gene silencing leading to a 'diploid' genome[11,28]. Similar extensive diploidization has also been observed in other organisms, including fish and amphibians[26].

New evidence suggests that changes in chromosome number brought about through polyploidy may also play a role in controlling gene expression, thus providing a novel form of epigenetic gene regulation[35]. For example, the expression of a single copy transgene introduced into *Arabidopsis* was reduced in triploid and tetraploid plants relative to diploids. Only one copy of the sequence was present, and thus the role of homology-dependent gene silencing could be ruled out. The precise mechanism involved was not determined, but a role for the chromosome number in regulating gene expression was strongly implicated[35]. Similarly, Guo *et al.*[36] examined RNA transcription levels for 18 leaf-tissue genes in monoploid, diploid, triploid and tetraploid

maize. The transcript levels for most of the genes were directly proportional to the structural gene dosage, but in a few cases gene expression was either greater or less than expected. In one gene, the transcript level was elevated more than sixfold in the triploid compared with the diploid or tetraploid. This unusual response, which is reminiscent of the 'odd/even effect' observed with B chromosomes in rye and maize[37], also suggests that chromosome number is involved in some way in controlling gene expression.

The evolutionary potential of being polyploid

Molecular studies reveal the polyploid nucleus to be a dynamic system, capable of undergoing considerable evolutionary change with respect to its diploid ancestors. Yet it seems that the extent and type of change, and thus the evolutionary potential of the polyploid, is influenced by several different, though possibly interacting, factors:

- The parental origin of the DNA sequences (paternal or maternal).
- The type of sequence (e.g. repetitive DNA or gene-coding DNA).
- The chromosomal location of the sequence (e.g. telomeric or centromeric).
- The position of the sequence in relation to its molecular environment (e.g. proximity to heterochromatin, promoter sequences and scaffold-attachment regions).
- The degree of genetic similarity between the different genomes being brought together in the polyploid nucleus.
- The level of ploidy in the plant.

The intergenomic chromosomal rearrangements that have been observed in several polyploids may represent one of a number of mechanisms that act by bringing together particular groups of genes into regions of the nucleus to enable these new types of sequence evolution, gene interactions and expression patterns to take place. Indeed, these changes may represent an important step towards the successful establishment of a newly formed polyploid.

Future prospects

Polyploidy is a fascinating phenomenon with intriguing evolutionary and practical potential. Given the high proportion of polyploids, and the high frequency with which new polyploids are formed, plants probably offer the best model system for new studies aimed at elucidating the molecular mechanisms and processes involved in polyploid formation and subsequent genome evolution. The absence of ethical problems, which affect the use of some animal systems, is another significant advantage. As so many economically useful species are polyploid, understanding the molecular mechanisms involved in diploidization will be vital for improved manipulation of their genomes to benefit humankind. Such improvement requires stable chromosome behaviour and controlled gene expression. Failure to achieve this would be a major factor limiting future advances in plant biotechnology. The first successful artificial crop species – *Triticale* – is an allopolyploid combining the genomes of wheat (*Triticum*) and rye (*Secale*)[38]. However, it is still impossible to predict which diploid genomes when combined will coexist stably and productively in new polyploids. Transforming the present 'hit-and-miss' process into an exact science based on molecular understanding would open the way forward for designing new and better polyploid species for agriculture, horticulture and silviculture.

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